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Personalized PDZ domains

Tracy Smith

How can researchers alter the cellular localization of a particular protein? The most common solution is to fuse a known localization sequence to the protein. However, not every subcellular compartment has a known localization signal and, therefore, alternative strategies are needed. Now, a paper appearing in the February issue of Nature Biotechnology (Schneider, S. *et al.*, **in the press**; 1999) reports that PDZ domains may provide useful platforms for developing new localization tools — in this case, PDZ domains with altered specificities targeted toward the C–termini of a wide variety of proteins with known cellular locations.

Many proteins contain protein interaction modules that allow them to cluster in specific complexes and perform particular functions, such as signal transduction. The ~100–residue PDZ domain is one such motif that interacts with the C–terminus of another protein. The name PDZ comes from the three proteins (post synaptic density–95, discs large, and zonula occludans, all members of the membrane–associated guanylate kinase family) in which this conserved motif was first recognized. PDZ domains have been divided roughly into two classes: those recognizing X–Ser/Thr–X–Val C–termini and those preferring C–termini ending in three hydrophobic residues.

Structural studies have shown that the ligand binds in a groove and that it extends an existing β–sheet on the PDZ domain by forming an additional β–strand. A C–terminal target peptide that is accessible to a PDZ domain and can bind in this manner is likely to be unstructured, not part of the core protein fold, and thus its sequence will be determined primarily only by necessary interactions with its PDZ domain

partner. For this reason, the range of allowed target peptide sequences is likely to be broad, and the PDZ domain architecture, in theory, should be an excellent scaffold on which to select new interactions.

In their paper, Schneider *et al.* demonstrate this experimentally. Starting with a common PDZ scaffold, they used a version of the yeast two–hybrid system to select PDZ domains that bind peptides with the following varied C–termini: Gly–Ser–Ala–Val, Gly–Lys–Tyr–Val, Thr–Arg–Tyr–Val, or Tyr–Tyr–Lys–Val. Of the four targets used for selection, two are random peptides and two are the C–termini of membrane proteins that have no known PDZ domain partners. The dissociation constant for each PDZ domain–peptide interaction is ~200 nM, similar to wild type PDZ domain–target affinities. These specific PDZ domains can be used to direct protein localization in mammalian cells.

Schneider *et al.* transiently transfected cells with constructs expressing one of the target membrane proteins and a PDZ domain–green fluorescent fusion protein, and visualized the cellular locations of the proteins by immunofluorescence and fluorescence microscopy, respectively. The fusion protein (top pair of images, right, in green) localizes to the membrane in the same pattern as its membrane protein target (top pair of images, left, in red). A control experiment (bottom pair of images) indicates that the localization is specific: a different fusion protein (right, green) containing a PDZ domain that cannot bind the C–terminus of the membrane protein target (left, red) does not localize to the membrane. The utility of such selected PDZ domains is likely to be high — they may be used to localize proteins, disrupt interactions, or bring proteins together into new complexes.